UNIVERSITI PUTRA MALAYSIA

POTENTIAL ROLE OF ENDOGEIC EARTHWORM IN MITIGATING BLOOD DISEASE IN BANANA PLANT

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FS 2016 14

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By

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Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

April 2016

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

POTENTIAL ROLE OF ENDOGEIC EARTHWORM IN MITIGATING BLOOD DISEASE IN BANANA PLANT

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April 2016

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Banana is an important fruit crop in many tropical countries including Malaysia. Besides the threat of Fusarium wilt and Moko disease, the occurrence of blood disease in Malaysia is another challenge to the cultivation. Therefore, there is an urgent need to search for effective treatment against the disease. Endogeic earthworms are known as soil fauna that improve soil properties and promote plant health, through their feeding, burrowing and casting activities. The present study aimed to evaluate the effect of *Pontoscolex corethrurus* (a common endogeic earthworm in the tropics) on blood disease in banana. Field sampling was conducted in a banana plantation infected with bacterial wilt disease. Identification of the causal pathogen was then carried out with morphological and molecular approaches. The pathogen obtained was confirmed to be blood disease bacterium (BDB). Secretions (cast and mucus) and soil inoculated with *P. corethrurus* were obtained to be tested on BDB *in vitro*. The results showed that earthworm mucus promoted the growth of *Pseudomonas* sp. that inhibited BDB. The interaction between *P. corethrurus*, BDB and banana was further evaluated through a glasshouse experiment. In the study, infected banana plantlets with earthworm inoculation showed less disease severity compared to un-inoculated ones, suggesting the role of endogeic earthworms in remediating blood disease. The finding was supported by nuclear magnetic resonance (NMR) and multivariate data analysis (MVDA) that revealed the presence of different groups of metabolites in the roots of the plantlets when inoculated with earthworms and BDB. The study suggested the potential role of endogeic earthworms (*P. corethrurus*) in enhancing soil microbial population and serve as possible bioremediation agent in plant disease management. Field trial and further investigations on the mechanisms that govern the changes are therefore essential to gain better understanding of this earthworm-BDB-host interaction.

Abstrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PERANAN POTENSI CACING TANAH DALAM MENANGANI PENYAKIT DARAH PISANG

Oleh

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Pisang merupakan tanaman buah-buahan yang penting kepada kebanyakan negara tropika termasuk Malaysia. Selain ancaman penyakit layu Fusarium dan Moko, kejadian penyakit darah menjadi satu lagi cabaran terhadap penanaman pisang di Malaysia. Oleh itu, usaha untuk mencari rawatan yang berkesan bagi menangani panyakit tersebut amat diperlukan. Cacing tanah dikenali sebagai fauna tanah yang memperbaiki sifat tanah dan meningkatkan kesihatan pokok dengan aktiviti pemakanan, pembentukan terowong dan penyahtinjaan. Kajian ini bertujuan untuk menilai kesan *Pontoscolex corethrurus* (sejenis cacing tanah yang biasa dijumpai di kawasan tropika) terhadap penyakit darah pisang. Persampelan dijalankan di sebuah ladang pisang yang dijangkiti oleh penyakit layu. Pengesahan terhadap penyebab penyakit dijalankan dengan kaedah morfologi dan molekul. Patogen telah disahkan sebagai *blood disease bacterium* (BDB). Rembesan (tinja dan mukus) dan tanah yang didiami oleh *P. corethrurus* digunakan untuk diuji kepada BDB secara *in vitro*. Ujian tersebut menunjukkan mukus cacing menggalakkan pertumbuhan *Pseudomonas* sp. yang menghalang pertumbuhan BDB. Kajian lanjut dalam rumah kaca dijalankan untuk mengkaji interaksi di antara *P. corethrurus*, BDB dan pisang. Kajian menunjukkan kehadiran cacing tanah dalam media tanaman telah mengurangkan tahap keseriusan penyakit darah dalam anak-anak pokok pisang. Ini menunjukkan potensi cacing tanah dalam mengurangkan kesan penyakit darah pisang. Dapatan kajian ini disokong oleh keputusan resonansi magnetik nuklear (NMR) dan analisis data multivariate (MVDA) yang menunjukkan kehadiran beberapa kumpulan sebatian kimia yang berlainan dalam bahagian akar anak-anak pokok apabila diinokulasi dengan cacing tanah dan BDB. Kajian ini mencadangkan potensi peranan cacing tanah (*P. corethrurus*) dalam meningkatkan populasi mikrob dan sebagai ejen bio-pemulihan dalam pengurusan penyakit tumbuhan. Kajian lapangan dan pemeriksaan lanjut terhadap mekanisma yang menyebabkan perubahan tersebut adalah penting untuk memperoleh pemahaman terperinci terhadap interaksi cacing tanah-BDB-hos.

ACKNOWLEDGEMENTS

I would like to extend my gratitude to my supervisor, Assoc. Prof. Dr. Nor Azwady Abd Aziz, who had provided a lot of guidance and assistance in my research and thesis writing. He has kept the research on track and at the same time allowed me to have much freedom in carrying out my research. Besides that, credits must also go to my co-supervisors, Assoc. Prof. Dr. Muskhazli Mustafa, Assoc. Prof. Dr. Intan Safinar Ismail and Dr. Rozeita Laboh who had provided constructive projections and facilities in their laboratories that allowed me to carry out the experiments without much hassle. Thank you for your generosity, time and experiences shared.

Supports were received by numerous people throughout the research. Laboratory experiments were made pleasurable with the kind supports from the laboratory assistants and research officers of Biology Department, Science Faculty, UPM and Malaysian Agricultural Research and Development Institute (MARDI). The research was sponsored by Malaysian Ministry of Education through the MyPhD program.

Special thanks to my friends, Sujithra Devi A/P Awinagiri Theva, Siti Rohani Sulaiman and Wong Wee Nee for their companion and help in the field and also in the laboratory. Last but not least, I would like to express my gratitude to my beloved family members and husband, Victor Lee who had showed unconditional care and support to me all the time. We are blessed to have our first born, Wallace Lee.

May God bless all of you.

I certify that a Thesis Examination Committee has met on 18 April 2016 to conduct the final examination of Teng Suk Kuan on her thesis entitled "Potential Role of Endogeic Earthworm in Mitigating Blood Disease in Banana Plant" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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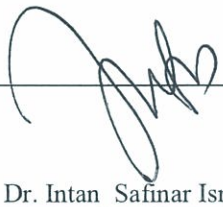
Signature: 
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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BDB	blood disease bacterium
cm	centimeter
CM	sterilized earthworm mucus
CPG	casamino acid-peptone-glucose
CS	control soil
EC	earthworm cast
EM	earthworm mucus
g	gram
g	gravity
Hz	hertz
kbp	kilo base pair
MHz	megahertz
mg	milligram
ml	milliliter
mm	millimeter
nm	nanometer
NMR	nuclear magnetic resonance
ppm	parts-per-million
rpm	revolutions per minute
SDW	sterilized distilled water
TPC	total phenolic content
TZC	tetrazolium chloride
µg	microgram
µl	microliter
µm	micrometer
v/v	volume/volume
WWS	worm-worked soil

CHAPTER 1

INTRODUCTION

Banana is one of the major fruit crops of the tropics and subtropics (Gantait *et al*., 2011). In Malaysia, banana is the second most important fruit crop after durian. There is approximately 27 500 ha of land cultivated with banana and this contributed to 11% of the total land cultivated with fruits in the country (Husain and William, 2011). It has an annual production ranging from 250 000 - 280 000 metric tonnes, value at more than RM400 million. Both cooking and dessert cultivars are grown in the country with Berangan and Cavendish banana accounting for approximately 50% of the total cultivars grown (Husain and William, 2011).

Pests and diseases are major constraints to the banana production in Malaysia (Tengku Ab Malik *et al*., 2010). Besides Moko disease, the recently detection of blood disease possess great threat to the banana cultivation in the country (Kogeethavani *et al*., 2013). Blood disease is another bacterial wilt disease affecting banana that is caused by blood disease bacterium (BDB) (Fegan and Prior, 2005). The disease was reported in 90% of the provinces in Indonesia and caused low banana production (Hadiwiyono, 2011; Hadiwiyono *et al.*, 2007). The symptoms of blood disease are similar with Moko disease and vary depending on the growth stages of host plant and mode of infection (Eden-Green, 1994). Confusion or misinterpretation of disease could occur if one is not familiar with the diseases. Blood disease is difficult to control due to lack of fundamental knowledge on the ecology and epidemiology of the disease (Hadiwiyono, 2011). With the invasion of blood disease in Malaysia, more studies are needed to gain knowledge on the possible approaches to control the disease. The threat posed by blood disease had generated an urgent need to develop ideally, a cost effective and environment friendly approach in curbing the disease.

In the field, the use of fungicides and insecticides, particularly the synthetic chemicals on disease control can only provide short term solution. In addition, it may raise concerns on the consequences of these chemicals on human health and environment. This has prompted the need to search for alternate, biological approaches for plant disease control. These approaches are preferably to be able to utilize the interactions among organisms in the environment and minimize the impacts to the ecosystem.

Earthworms are known as soil engineers due to their activities that change the physico-chemical and structure of soils (Jones *et al.*, 1994). Their activities affect soil microorganisms, soil organic matter (SOM) regulation and plant growth (Meghvansi *et al.*, 2011). Earthworms are also found to increase the concentrations of various metabolites in plants (Lohmann *et al.*, 2009; Wuyts, 2006; Wurst *et al.*, 2004). Some of these compounds are involve in the natural defense mechanism in plants. Their concentrations would change in response to pathogenic infection (Lohmann *et al*., 2009). Previous studies had documented the ability of *Pontoscolex corethrurus* in reducing the damage caused by root-feeding pathogens (Loranger-Merciris, *et al.*, 2012; Lafont *et al*., 2007; Blouin *et al*., 2005). As soil burrowers that have close relationship with the soil environment and plant system, *P. corethrurus* may serve as potential disease remediating agent. This in turn would promote plant health and increase its resistance towards diseases. However, there is limited knowledge on this earthworm-

plant interaction in controlling plant pathogens. Further studies are therefore important to gain more insight on this interaction.

1.1 Problem statement

Plant diseases are the major concern and lead to economic threats to the agricultural sector. In the attempt to control plant pathogens, pesticides are applied when the symptoms are visible, which are often too late to be effective *(*Ghorbani *et al*., 2009). The use of pathogen resistant crops serves as an alternative as opposed to the usage of chemical pesticides. However, these plant varieties are usually scarce and do not meet the cultural standards (Wuyts, 2006). Therefore, a better approach will be concentrating on the prior-infection period and improving soil conditions that are favorable to the plants. Plants will be able to grow better and are less susceptible towards diseases with the improved soil and environmental conditions.

Besides the threat of Moko disease and Fusarium wilt, the occurrence of blood disease possesses another great challenge to the banana cultivation in Malaysia. The host range for Moko disease is very broad whereas blood disease is confined to banana (Álvarez *et al*., 2010; Denny, 2006). The identification of causal agent is therefore important to search for control and cultural practices that are specific and effective to the disease.

Blood disease is one of the major disease in banana that occurs in Indonesia and, currently there is still no effective treatment against the disease (Hadiwiyono *et al.*, 2013). The disease is soil-borne and can be transmitted by insects and human activities (Hermanto *et al*., 2013). Blood disease is highly virulent and has affected 27 out of 30 provinces in Indonesia (Hadiwiyono *et al.*, 2007). In view of the severe loss experienced by Indonesia, the search for effective and appropriate approach to be applied in the field is highly desired to mitigate the impact of blood disease. Knowledge and the advancement of controlling approach on blood disease are also useful to banana plantain infected with Moko disease, as the mode of infection and spread of both diseases are very similar.

Soil burrowing earthworms are known to have close relationship with plant root system and affect above and belowground organisms. However, knowledge on the effect of earthworms on the production of metabolites that are involved in plant defense mechanisms remains unclear. Such knowledge is important in understanding the contributions of earthworms on plant health and soil environment that help to maintain the ecological balance. It may also serve as potential mode of therapy for infected plant and soil systems in sustainable agriculture.

1.2 Objectives and research outline

The present study is carried out with the aim to determine the possible contributions of endogeic earthworm, *P. corethrurus* on blood disease in banana. The following objectives are developed to achieve the aim:

- To determine the causal agent of bacterial wilt disease in banana through morphological and molecular identifications

- To evaluate the effect of earthworm mucus, worm-worked soil and cast on blood disease bacterium (BDB) *in vitro*
- To determine the effect of earthworm on BDB and its host plant
- To evaluate the effect of earthworm and BDB on the metabolic changes in banana

The general research outline of the study is depicted in Figure 1.1.

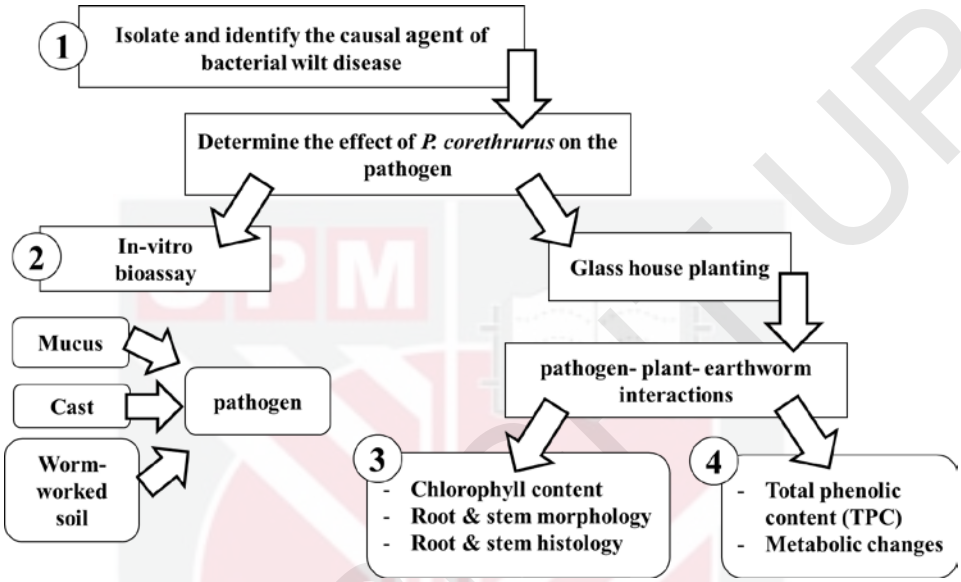

Figure 1.1: Research outline of the study

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